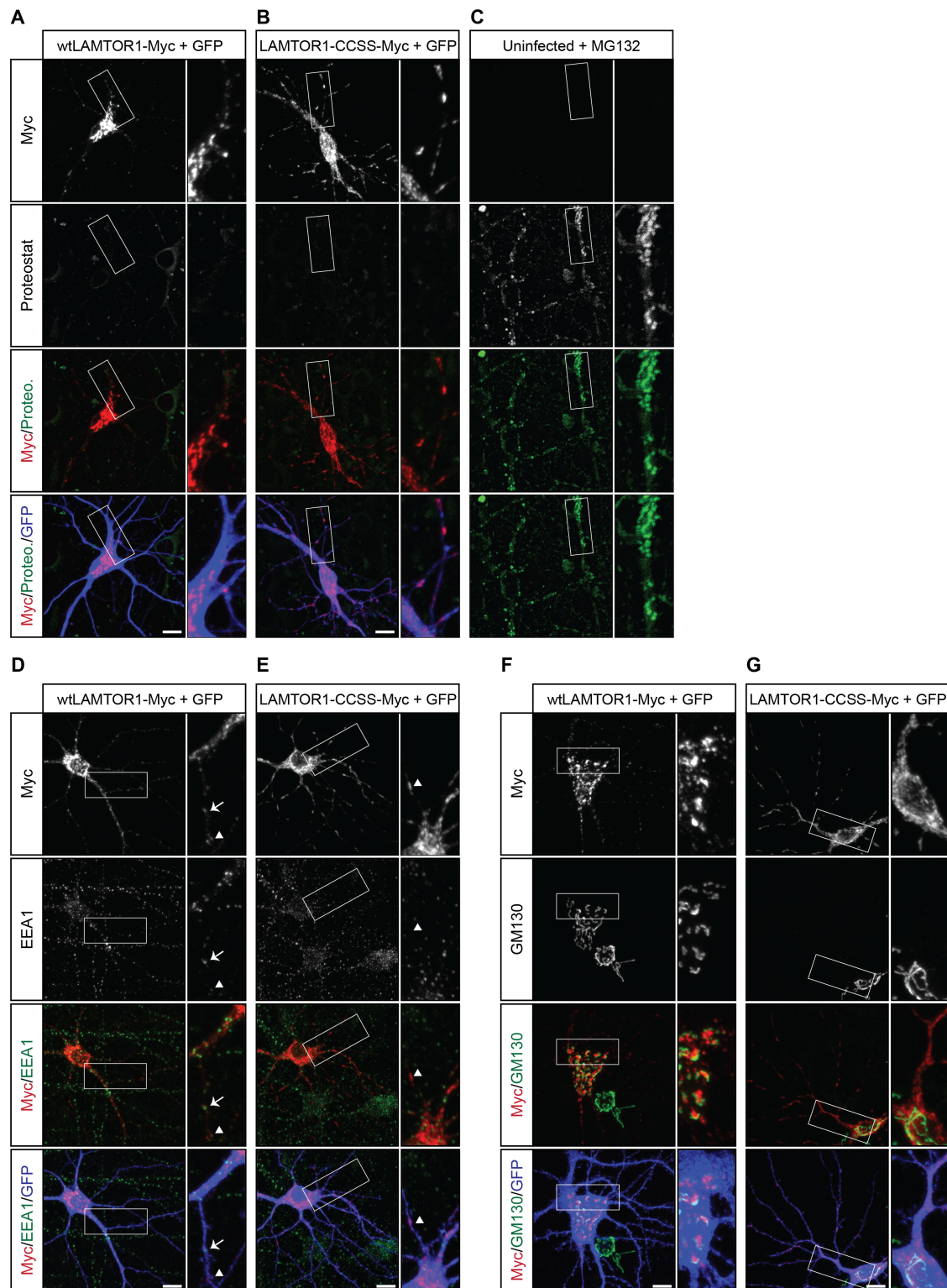
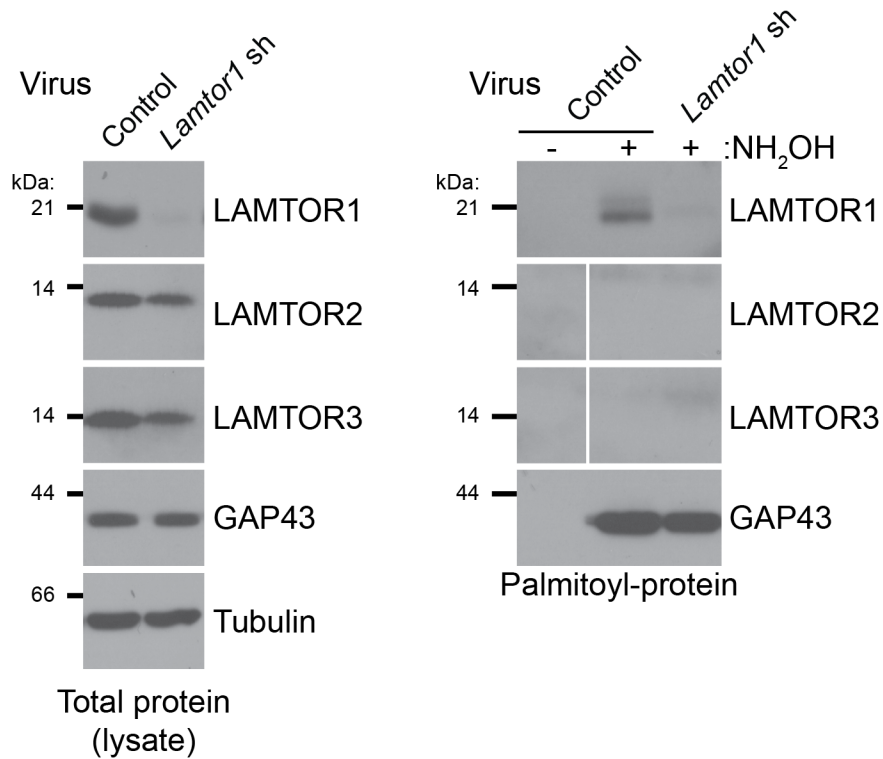


**Supplemental Figure 1. mTORC1 activation by amino acids is palmitoylation dependent in HeLa cells.** HeLa cells were stimulated with amino acids using the same paradigm as for HEK293T cells (Figure 2A). Cells were incubated in serum free media 16-20 hours (overnight) prior to being placed in Earle's Balanced Salt Solution (EBSS). One hour and 45 minutes later cells were treated with 20  $\mu$ M 2BP or ethanol (EtOH, vehicle control) for 15 min, prior to stimulation with 2x MEM amino acids (AA) or vehicle for 30 minutes. Lysates were blotted to detect phospho-S6 (p-S6, top), S6 total (middle), and tubulin (bottom) levels. Similar results were observed in a replicate experiment. Panels are composites of the same Western blot image.



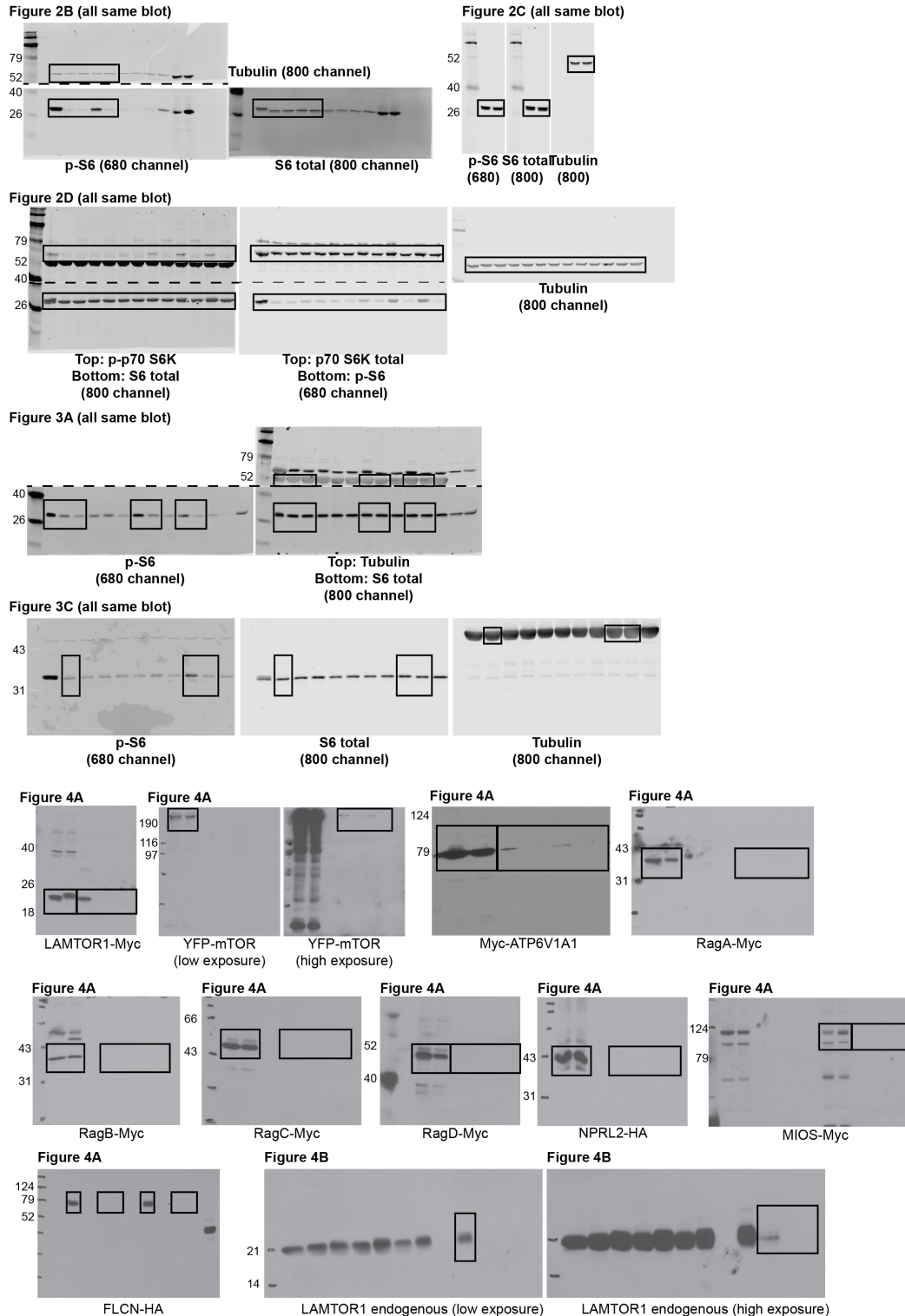
**Supplemental Figure 2. wtLAMTOR1-Myc occasionally localizes to early endosomes but not to the Golgi and neither wt or CCSS LAMTOR1-Myc forms aggregates. (A).** *First column:* images of Myc and the aggresome detecting dye Proteostat and merged images of Myc/Proteostat (Proteo.) and Myc/Proteo./GFP signals from hippocampal neurons transfected to express wtLAMTOR1-Myc and GFP and

incubated with Proteostat after fixation. *Second column*: magnified views of boxed area of first column images. **(B)** As in A except for neurons transfected to express LAMTOR1-CCSS-Myc and GFP. **(C)** As in A and B, except for untransfected hippocampal neurons treated with MG132 to block proteosomal degradation and induce protein aggregation. **(D)** *First column*: images of Myc and early endosome marker EEA1 signals and merged images of Myc/EEA1 and Myc/EEA1/GFP signals from hippocampal neurons transfected to express wtLAMTOR1-Myc and morphology marker GFP. *Second column*: magnified views of boxed area of first column images. Arrows indicate LAMTOR1-Myc positive vesicles that are also EEA1 positive, arrowheads indicate LAMTOR1-Myc puncta that are EEA1 negative. **(E)** As in D except for neurons transfected to express LAMTOR1-CCSS-Myc and GFP. **(F)** and **(G)** as is in D and E, respectively, except using the Golgi marker GM130. Scale bar indicates 10  $\mu$ m.



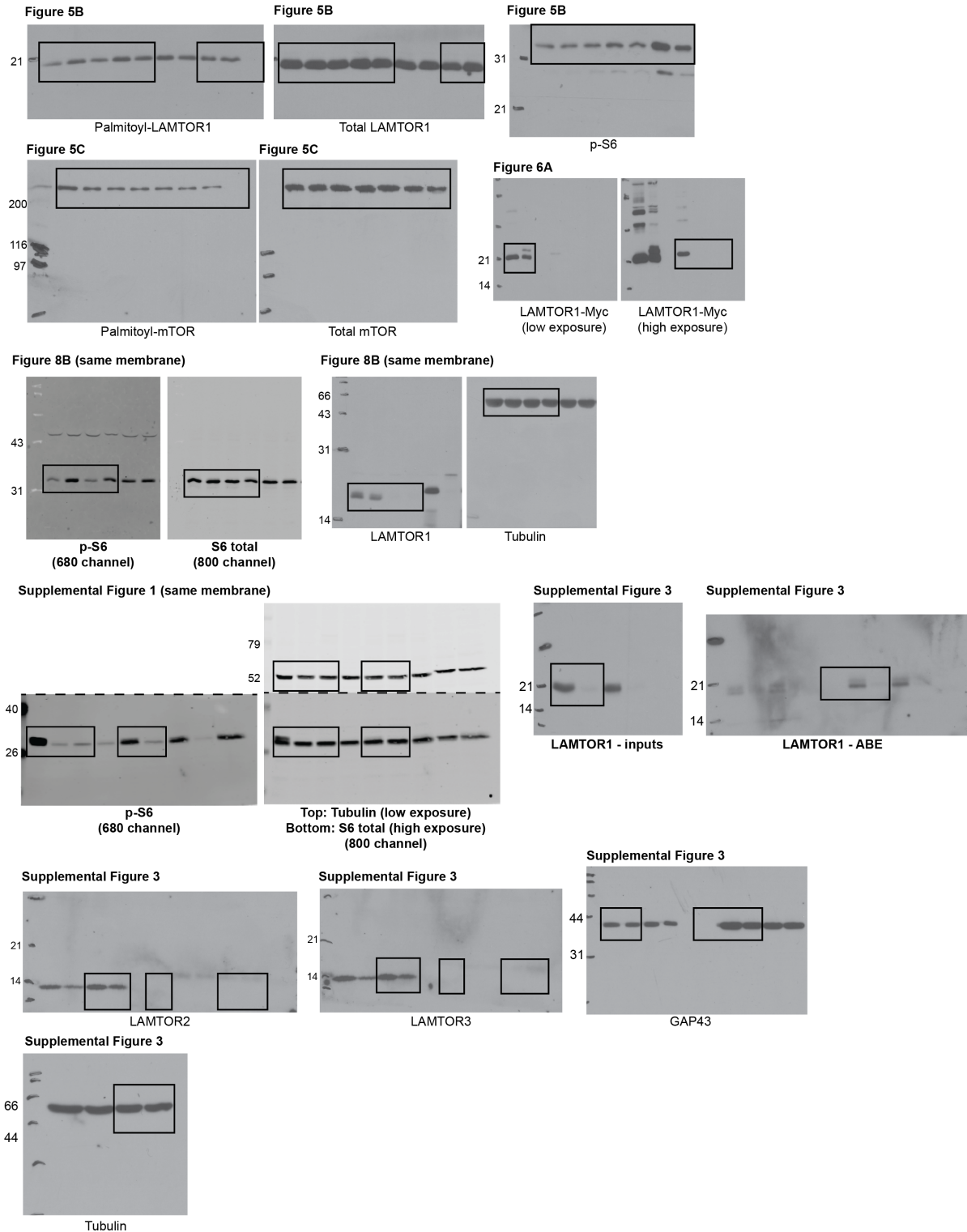
**Supplemental Figure 3. Knockdown of LAMTOR1 does not trigger palmitoylation of LAMTOR2 or LAMTOR3 in hippocampal neurons.** (A) Total expression (left panels) and palmitoyl-levels (right panels) of LAMTOR1, LAMTOR2, LAMTOR3, GAP43, and tubulin isolated from control or *Lamtor1* shRNA infected hippocampal neurons. Panels are composites of the same Western blot image.





**Supplemental Figure 4. Uncropped Western blot images for Figures 2, 3, and 4.**

Boxes indicate cropped regions and dashed lines indicate where membranes were cut.



**Supplemental Figure 5. Uncropped Western blot images for Figures 5, 6, 8, and Supplemental Figures 1 and 3. Boxes indicate cropped regions and dashed lines indicate where membranes were cut.**